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On two endozoic trichodinids, *Trichodina urinaria*
Dogiel, 1940 and *Trichodina polycirra* sp. n.

(Contribution to the knowledge of trichodinids, III)

O dwóch endozoicznych trichodinach, *Trichodina urinaria*
Dogiel, 1940 i *Trichodina polycirra* sp. n.

The trichodinid parasites of Czechoslovak fish have been very little known hitherto, and only a few papers were dealing with the European representatives of this group living on fresh-water fish. Only in latest time there appear more papers dealing with these ciliates. The aim of the present paper is to fill partly the gap in our knowledge of the most conspicuous among these parasites, namely, the endoparasites of urinary bladders of our fish. For the time being, two species were found: *Trichodina urinaria* Dogiel, 1940 in *Perca fluviatilis* and *T. polycirra* sp. n. in the urinary tract of *Rutilus rutilus*. In the former species, a redescription was necessary because of the lack of an adequate description of the body shape, adoral zone and especially of the adhesive disc; the latter species is established as a new one, since it differs very distinctly from all other endoparasitic trichodinids.

Trichodina urinaria Dogiel, 1940

After Dogiel's original description of this ciliate, it was found again by Stein (1954) in *Perca fluviatilis*. In Czechoslovakia the first report is that of Vojtek, Folkmanová et Ergens (1954), who found this form abundantly in perches, too. Moreover, Ergens found this ciliate in the urinary tract of *Lucioperca lucioperca*; his material was handed over to further study to Vojtek (1954). During the course of study of parasites of our fish *T. urinaria* was found in perches from three localities. The in-

tensity of infection fluctuated, but in general it was always more heavy in grown up fish than in young ones (up to 15 cm). The infection being heavy, the ciliates invade the ureters. The extensity of infection was about 50 %.

Existing descriptions of this ciliate were dealing before all with biometrical evaluation of the adhesive disc, but the morphology as well as the structure of the adhesive disc after Ag-impregnation, so important for the specific differentiation in this group, remained unknown. We offer, therefore, a redescription of this species.

Table I
Survey of data on *Trichodina urinaria* Dogiel and *T. polycirra* sp. n.

Data	<i>T. urinaria</i> Dogiel	<i>T. polycirra</i> sp. n.
Host	<i>Perca fluviatilis</i>	<i>Rutilus rutilus</i>
Locality	surroundings of J. Hradec in Bohemia	surroundings Trebon in Bohemia
Habitat	urinary bladder and ureters	urinary bladder and ureters
Diameter of body	68 μ (60–74)	67 μ (58–71)
Diam. of adhesive disc	45 μ (41–50)	52 μ (48–58)
Diam. of denticulate ring	25 μ (21–29)	38 μ (28–45)
Diam. of the arch of macronucleus	40–66 μ	20–29 μ
Number of denticles	36(29–40)	42(38–55)
Number of radial pins to one denticle	6(7)	5–6
Dimensions of a denticle	thorn 4–6 μ , blade 6 μ , width of the central part 2 μ , total length 5 μ	thorn about 2.6 μ , blade about 4.8 μ , width of the central part 1.7 μ , total length 5 μ
Velum	broad (5 μ) and thick	weakly developed
Marginal cilia	stout, in wide intervals	unusually numerous
"Height" or length of the body	about 25 μ in unatta- ched animal	about 20 μ in unatta- ched animal
Micronucleus	?	?
Border membrane	about 4 μ wide	about 3 μ wide
The adoral zone performs	a 540° spire	just less than 360°

The principal data of this ciliate are summarised in Tab. I. The body of a free-swimming ciliate in a normal physiological state is

circular in aboral aspect, in side view it has the shape of a loaf of bread or it is hat-shaped (Fig. 1). Quite remarkable is the velum which is in shape of a rather broad (5 μ) and thick ring, giving in side view the idea of a hat-brim. The velum has an undulated edge, it is a very delicate structure capable of different movements and

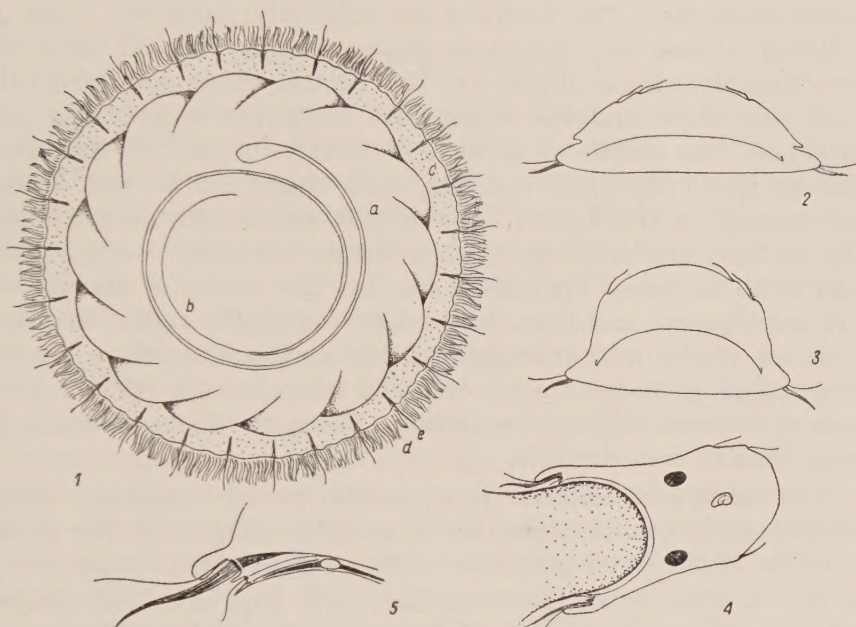


Fig. 1. *Trichodina urinaria* Dogiel. 1 — a view of the adoral face of the ciliate, with oblique furrows. a = one and half turn of the adoral zone, b = prolongation of the adoral zone, c = velum, d = locomotory ciliary girdle, e = marginal cilia. 2 — side view of a swimming *Trichodina*. 3 — a ciliate attached to the substrate; in both the inverted plane of the adhesive disc is indicated. 4 — a section through *Trichodina* attached to a villus of an ureter. 5 — a section through the border part of the body, with the inner and outer ciliary girdle separated by a septum, just above the border membrane. Just above the locomotory outer girdle of membranelles is the thick velum with marginal cilia, inserted into incisions.

contractions. After the death of the ciliate it instantaneously loses its normal shape. On its bottom side there are in the living ciliate distinct narrow incisions, in which the stout marginal cilia are inserted. The oral surface of the body is distinctly furrowed by oblique grooves, their number being about 13. Trichodinids, attached

to the epithelium of the urinary bladder or to the villi of ureters, may assume quite another shape — in most cases they are strongly vaulted and elongated in their oral-aboral axis, their adhesive disc being considerably inverted inwards, to form a deep sucker by means of which the body is attached to the substrate.

The adhesive disc is encircled by a circa $4\ \mu$ wide, finely striated border membrane. The denticles are relatively numerous (Tabl. I) in living ciliates only the thick strong central parts of denticles are distinctly visible (Phot. 1). The central cones are relatively broad and short and the bases both of thorns and blades are thickened. The length of denticle is relatively short if compared with its span. After impregnation, their shape can be well observed; because of their great number they are densely inserted one into another, and their thorns and blades are narrow and almost even as in no other *Trichodina* species. The centre of the disc is dark impregnable, and from this background clearly reflect the rests of thorns of the mother-individual denticles; unlike other *Trichodina*, the old denticles are not resorbed fully, but the rests of their thorns persevere till the new division. There are 6 (exceptionally 7) radial pins to each denticle.

Just above and anterior (oral) to the border membrane there are two ciliary girdles, separated by a septum (Fig. 1, 5). The membranelles of the outer girdle are rather massive, their bases being about $3\ \mu$ wide. From kinetosomes of both girdles extend distinct fibrils to the centre of the adhesive disc. They play certainly an important role in the contractility of the disc.

The adoral zone performs a spire of about 540° , i. e., one and a half turn. Two rows of stout and short cilia follow their course and are continued through the mouth into the cytopharynx. The cilia are closely adjacent to the oral face of the animal. After Ag-impregnation a sort of continuation of the zone is demonstrated in most animals, performing another half-turn, but always devoid of cilia. The nature of this prolongation could not be discerned, however, it does not seem to consist of kinetosomes.

The macronucleus is horse-shoe shaped, or more conveniently, it has the shape of an unclosed circle, the outer diameter of which ranges from 40 to $66\ \mu$, and is about $7\ \mu$ thick. We could not (like Vojtek, 1957) discover the micronucleus in most animals; only in dividing animals it could be observed as a spindle-shaped body

with formed chromosomes. The contractile vacuole is placed slightly excentrically.

Our findings corroborate in general the data of earlier workers (Dogiel, 1940; Stein, 1954; Vojtek, 1957), their slight diversity not exceeding the normal variaton range and prove *T. urinaria* to be a well founded species.

Trichodina polycirra sp. n.

This species was found in the urinary tract of *Rutilus rutilus* on two localities in the surroundings of J. Hradec in southern Bohemia. Thanks are due to Dr. R. Ergens who drew our attention to the occurrence of this ciliate. About 30% of *Rutilus rutilus* were found to be infected on the locality, the intensity of infection being in most cases very low, especially in young fish. In heavily infected fish the ciliate ascend the ureters.

T. polycirra sp. n. differs at first sight from *T. urinaria*. In side view the body of unattached ciliate is not so high (Fig. 2, 3). The oral, convex face of the animal is furrowed in a characteristic manner: towards the border of the body disc there extend also oblique grooves, but they are more numerous (about 20) and more shallow than in the preceeding species. They make the circular outline notched in oral aspect. The course of the adoral zone is rather peculiar, too (Fig. 2, 4). In most cases it makes almost one complete turn, only exceptionally it surpasses by a little 360°. The zone is as almost regular circle, only the spot where the gap is between the beginning of the zone and its end it is slightly pressed inwards. In many cases the distance between the beginning of the zone and the vestibulum is quite considerable. From the circle of the adoral zone there extend numerous fine furrows in a straight centripetal direction; the centre of the oral surface is smooth.

The adhesive disc, in unattached animal rather flat, can be considerably inverted inwards in animals adhering to the substrate. It is encircled by a 3 μ wide border membrane, finely striated. The denticulated ring consists of a great number of denticles — average 42 (38—55), thus attaining one of the highest values in *Trichodina*. In living ciliate, only the central parts of denticles are discernible, whereas blades and thorns, owing to their fineness, are almost invisible. The central parts of denticles are also relatively wide and short, as in *T. urinaria*, but of another structure:

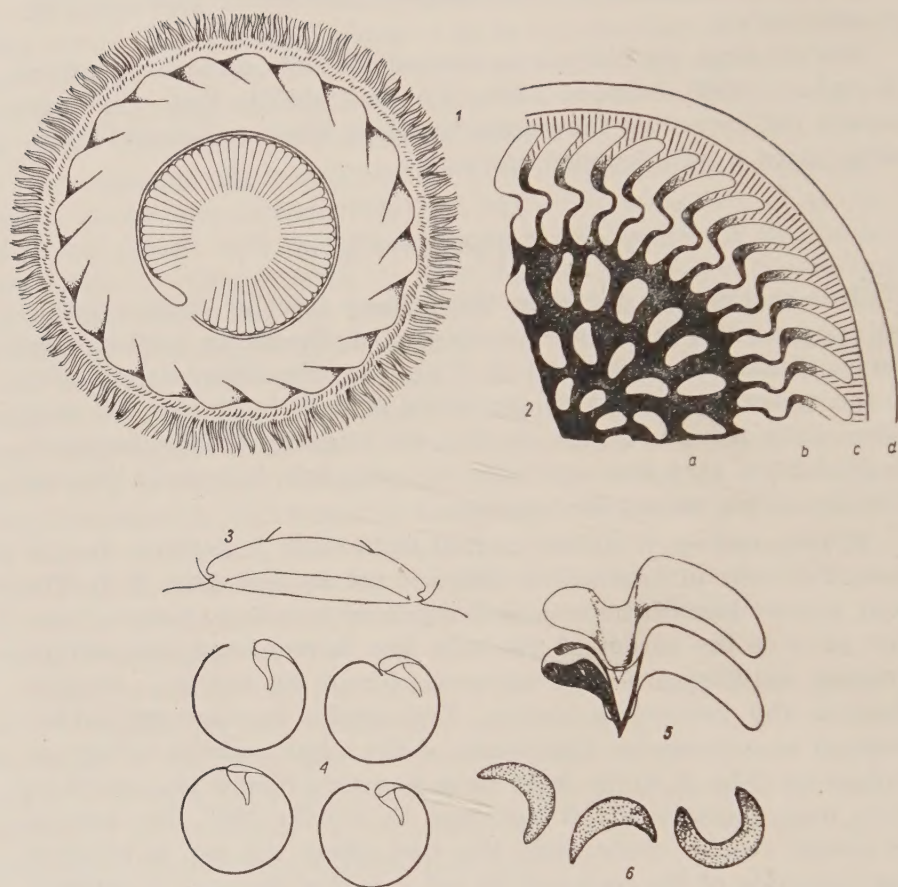


Fig. 2. *T. polycirra* sp. n. 1 — the adoral face with numerous oblique grooves, uncompleted circle of adoral zone and fine radial furrows inside it. 2 — Sector of an impregnated adhesive disc of *T. polycirra* sp. n. a = rudiments of old denticles, arranged into three circles, b = denticulate ring, c = radial pins, d = border membrane (its fine striation not indicated). 3 — side view of a free-swimming animal. 4 — four different pictures of development of adoral zone. 5 — two denticles; the above one in surface view, as seen after Ag-impregnation, the bottom one in optical view as seen in living ciliate. 6 — three different pictures of macronucleus.

the central cone proper, through which the denticles are inserted one into another, is relatively fine, only its inner arm reveals a considerable thickness at the place where it passes into the thorn

(Fig. 2, 2). The crooked blades are similar to those in *T. urinicola*, but the thorns are of a rather peculiar form, being very flat and short but of a considerable width and are also slightly crooked backwards. In the dark impregnable centre of the adhesive disc, the remnants of two or three preceding sets of denticles can be demonstrated by Ag-impregnation, which denticles were resorbed after the new denticulate ring had been formed in the daughter individual. These remnants of thorns of the old denticles are situated in three or two circles, the inner one is the oldest; the number of these remnants is reduced towards the centre to approximately one half of the preceding circle. There are 5 to 6 radial pins to one denticle.

The two aboral ciliary girdles are arranged in the common way. The marginal cilia are situated beneath an insignificantly developed velum, they are unusually numerous, not too long but rather strong, and curve upwards past the side walls of the ciliate.

The macronucleus is in most cases crescent-shaped, only sometimes in form of a horse-shoe, has rather irregular outlines and more or less pointed ends. Its outer diameter is 20—29 μ . Micro-nucleus could not be found neither in preparations stained by Feulgen nor in those stained by iron haematoxylin. It was neither possible to distinguish it in several division stages stained according to Feulgen. We suppose it is perhaps quite closely adjacent to the macronucleus, most probably in an excavation of the latter. The contractile vacuole is situated excentrically near the mouth vestibulum.

From a comparison with other hitherto described *Trichodina* species it is clearly evident that the ciliate just described is a new species. We propose for it the name *T. polycirra*. It is impossible to compare it with any of the ectoparasitic species because of great morphological differences, so that we have to differentiate it only from the endoparasitic ones. From *Vauchomia renicola* and *V. nephritica* it differs in having only one turn of the adoral spiral, not to mention other differences. From all other hitherto known endozoic species from fish (*T. urinaria*, *T. alburni*, *T. fariai*) it differs by much greater number of denticles. Only two other trichodinids are reported up to this time to be furnished with such a great number of denticles, the unsufficiently described *T. bufonis* Fant-ham and *Vauchomia renicola* Mueller. The further characteristic features of this species (and in the same time differences as

compared with both *T. urinaria* and *T. alburni*) are the short and extremely broad thorns of denticles, the peculiar course of the adoral zone and the unusually numerous marginal cilia (= cirri, *polycirra*).

Several years ago, Stein (1954) found a *Trichodina*-infection of the urinary bladder of *Rutilus rutilus*, which she regarded as different from *T. urinaria*. However, we can not believe her finding was identic with *T. polycirra*, because the number of denticles was relatively low, ranging from 33 to 36.

Discussion

The two described ciliates are interesting from several points of view. The very long adoral zone of *T. urinaria* reveals a certain kinship with the genus *Vauchomia* in those individuals in which can be observed a continuation of the 540° adoral spiral in form of a trace without cilia. Thus *T. urinaria* can be perhaps listed as transitional form between the genus *Vauchomia* and other endozoic trichodinids (*T. polycirra*, *T. alburni*). Most probably the adoral zone of *T. urinaria* is now in a state of regression, and the irregularly occurring rudiments of much longer adoral zone could be taken for a testimony of an originally long (720° , two turns) zone. As a regressive character of the zone in *T. urinaria* could be perhaps explained even the fact, that its cilia were always observed to protrude motionless (in undamaged ciliates) and a probably osmotroph nutrition (no solid content in the digestive vacuoles).

Owing to the importance attached to the length of the adoral zone in the systematics of Urceolarids, we have to take Mueller's genus *Vauchomia*, which displays two to three turns of the adoral zone, for a good, duly founded genus, and not only subgenus, as some later authors, and we ourselves (1958), did. *T. urinaria*, although it has a very long spiral, does not yet comply with the generic characteristic of *Vauchomia* and cannot be placed into this genus, its adoral zone representing only a degree of transition between *Trichodina* and *Vauchomia*.

On the other hand, there exist some traces of transition — as for the development of the zone — also between *T. polycirra* (adoral zone in many cases remarkably less than 360°) and many *Trichodinella* [e. g., *Trichodinella (Tripartiella) bulbosa* (Davis)]. A whole series could be placed together from representatives of *Urceolarii*-

dae, furnished with *Trichodina*-like skeletal elements, according to the length of their adoral spiral: *Semitrichodina sphaeronuclea* — much less than 180° , *Trichodinella* (T.) *epizootica* — about 180° , *Tripartiella* (T.) *bulbosa* — about 270° , *Trichodina polycirra* sp. n. — less than 360° , different ectoparasitic *Trichodina* — 360° and more, *Trichodina urinaria* — 540° , *Vauchomia* — 720° and more. This gradual development of the adoral ciliary apparatus should not be taken as a testimony against the appurtenance of the ciliates in question into different genera (as they exhibit many other different features), it only emphasizes the inadvisability of dogmatic insistence upon the existence of clear-cut lines between closely related genera.

The problem remains, what degree of the development of the adoral zone should be taken for the phylogenetically original one. From the standpoint of the ingenious theory, pronounced also by Corliss (1956), according to which the adoral zone developed by an enormous prolongation and spiralisation of the undulating membrane and adoral zone of membranelles of thigmotrichous ciliates, the most original should be the short zone of *Trichodinella*, that one of *Vauchomia* being the latest step in development. However, one could hardly suppose such great development of the adoral zone would be further functional in an endoparasitic genus, living in an environment, where the driving of food to the mouth is bound with much less effort than in free water. The final solution of the relation between phylogenetic development and that one of the adoral zone is to be postponed, in our opinion, to the time when the morphogenesis of this group and its further representatives will be better known.

T. urinaria and *T. polycirra* show in their morphology many common features with endoparasitic trichodinids from amphibians (great number of denticles, their narrow blades, possibility of cup-like inverting of the adhesive disc etc.) but there is one striking difference, the occurrence of stout marginal cilia in species from fish. Although the occurrence of these cilia is not so important as the structure of the adoral zone, yet its lack in the species from amphibians could make justifiable their intrageneric separation. As late as 1928, Zick created a new genus *Acyclochaeta* for trichodinids without the girdle of marginal cilia, e. g., *T. steini*, *T. baltica*, *T. bidentata*, *T. patellae*, *T. urinicola*. Nowadays, we know that all the first three species possess marginal cilia, so that they do not

comply with the diagnosis of *Acyclochaeta*, *T. patellae* is a true *Urceolaria*, only *T. urinicola* and other species from amphibians are really devoid of marginal cilia. Therefore we patronize the opinion that the taxon *Acyclochaeta* be preserved, although we adopt a conservative view and propose that it be accepted as a subgenus of *Trichodina*. For this reason we propose to designate this species as *T. (Acyclochaeta) urinicola* (Fulton).

Another morphological peculiarity is represented by the rudiments of thorns of old denticles, which are preserved even after a new denticulate ring was formed in the daughter individual. In *T. urinaria* these rudiments endure only one following division, the situation thus being similar to *T. unionis* Hampl (see Phot. 4) or *T. macommarum* J. & Z. Raabe, but in *T. polycirra* we find in the centre of the adhesive disc rests of three "generations" of worn-out denticles.

The biology of these ciliates remains still an enigma. The mode of transmission is unknown, but since encystment in this genus is unknown, it seems probable that the ciliate must at times leave the host and pass into the water and either find a new host or perish. The probability of penetrating into a new host is certainly very small, if we consider the short time (maximum 20 min.) the parasite remains undamaged in pond water. The infection takes place without doubt per cloacam, and certainly not only during the spawning time (Mueller, 1938) as also young *Perca* and *Rutilus* harbor trichodinids, although in very little number. Probably some trichodinids are always present in the urine discharged from the infected fishes.

The presence of numerous parasites in the bladder seems to produce no serious damage to the host. We can find a large mass of cellular débris in the infected bladders — as we observed it formerly in amphibians (Lom, 1958) — but the fish showed no retardation of growth or another sign of trouble. This débris originates under the "sucking" activity of the ciliate's adhesive discs, by which the epithelial cells are loosened and sloughed away into the lumen. The irritation caused by the ectoparasitic species on the fish's skin is either not so effective or the loosened cells are lost in the slime normally covering the skin, but in the bladder this desquamation is very striking. Although the trichodinids ascend the ureters as far forward as the width of the lumen will permit, passing by this way sometimes into the posterior part of the kidney,

this organ appears normal, shows no marks of inflammations. Certainly the parasites have been long and well adapted to their mode of life and to their hosts.

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STRESZCZENIE

Autor podaje wyniki swych badań nad endozoicznymi gatunkami rodzaju *Trichodina*, występującymi u ryb Czechosłowacji, a mianowicie *Trichodina urinaria* Dogiel, 1940 i *T. polycirra* sp. n. U pierwszego z tych gatunków z pęcherza moczowego okonia autor stwierdza występowanie spirali adoralnej rzęsek, zataczającej łuk ok. 540° , a zaczynającej się jeszcze dodatkowym łukiem ok. 180° , pozbawionym rzęsek. *T. urinaria* nawiązuje więc do przedstawicieli rodzaju *Vauchomia* Mueller, opisanych z pęcherza moczowego szczupaków Ameryki Północnej; ze względu jednak na mniej rozwiniętą niż u *Vauchomia* spiralę adoralną autor utrzymuje gatunek *T. urinaria* w obrębie rodzaju *Trichodina*. U *T. polycirra* sp. n. z pęcherza moczowego płoci autor stwierdza swoistą budowę spirali adoralnej i tarczy czepnej, dobrze wyodrębniającą ten gatunek od innych gatunków rodzaju *Trichodina*.

W dyskusji autor omawia prawdopodobne znaczenie różnego stopnia rozwoju spirali adoralnej, szczególnie u form endozoicznych, budowę środkowej części tarczy czepnej, na której dopatruje się istnienia resztek rozpuszczonego rodzicielskiego wieńca haków, oraz zagadnienie patogenności pasożytów pęcherza moczowego ryb.

EXPLANATION OF PLATES

PLATE I

Phot. 1. An unfixed adhesive disc of *T. urinaria* Dogiel. Only the central parts of denticles can be seen, radial pins and striation of the border membrane.

Phot. 2. An impregnated adhesive disc of *T. urinaria* Dogiel.

PLATE II

Phot. 3. The adhesive disc of *T. polycirra* sp. n. in fresh preparations. Of the denticulate ring, only the fine central cones and thickenings of thorns are visible.

Phot. 4. The adhesive disc of *T. unionis* Hampl.

